

WHAT IS CLAIMED:

1. A method for making a peptide-carrier conjugate, comprising:
modifying a first peptide to produce a second peptide so that the pI of the second peptide
5 is in a favorable range or closer to the range than the pI of the first peptide; and
conjugating a plurality of the second peptide to an OMPC to obtain a peptide-carrier
conjugate;
wherein the peptide load, or the solubility of the conjugate, or both of them are increased
by the modification; wherein the second peptide has a non-naturally occurring sequence.
10
2. The method of claim 1 wherein the pI of the second peptide is lower than the pI
of the first peptide.
3. The method of claim 1 wherein the first peptide has an amino acid sequence
15 selected from a pathogen.
4. The method of claim 3 wherein the pathogen is selected from the group
consisting of *Haemophilus influenza*, hepatitis viruses A, B, or C, HIV, human papilloma virus, measles,
mumps, rubella, varicella, rotavirus, *Streptococcus pneumonia* and *Staphylococcus aureus*.
20
5. The method of claim 1 wherein the first peptide has a sequence selected from the
amino acid sequence of HA protein of Influenza virus A or B, or HIV gp41 protein.
6. The method of claim 1 wherein the first peptide is modified at or near either
25 terminus of the first peptide sequence.
7. The method of claim 1 wherein the first peptide is modified by adding at least
one amino acid with anionic side chain to the first peptide sequence.
8. The method of claim 7 wherein the at least one amino acid with anionic side
30 chain is Glutamate or Aspartate or both.
9. The method of claim 1 wherein the first peptide is modified at the side chain of
an amino acid residue in the first peptide sequence.
35

10. The method of claim 9 wherein the modification at the side chain is selected from the group consisting of phosphorylation or sulphation of serine, threonine, tyrosine, aspartate, or histidine, carboxylation of glutamate, acylation of lysine, or oxidation of cysteine.

5 11. The method of claim 1 wherein the first peptide is modified at N-terminal or C-terminal of the first peptide sequence.

12. The method of claim 11 wherein the first peptide is an amidated peptide and is modified with carboxylation of the C terminal group.

10 13. The method of claim 11 wherein the first peptide is modified with acylation of N-terminal amino group.

14. The method of claim 13 wherein the acylation is acetylation or succinylation.

15 15. The method of claim 1 wherein the plurality of the second peptide are conjugated to the carrier using an agent selected from the group consisting of sulfosuccinimidyl 4-(N-maleimidomethyl)cyclohexane-1-carboxylate (sSMCC), N-maleimidobenzoyl-N-hydroxysuccinimide ester (MBS), glutaraldehyde, 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDCI), Bis-diazobenzidine (BDB), or N-acetyl homocysteine thiolactone (NAHT), N-maleimidobenzoyl-N-hydroxysuccinimide ester (MBS), glutaraldehyde, 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDCI), Bis-diazobenzidine (BDB), and N-acetyl homocysteine thiolactone (NAHT).

25 16. The method of claim 15 wherein the agent is sSMCC.

17. The method of claim 1 wherein the first peptide has a molecular weight at the range from 500 Da to 30000 Da.

30 18. The method of claim 1 wherein the first peptide has a molecular weight at the range from 1400 Da to 7900 Da.

19. The method of claim 1 wherein the peptide load is increased to more than 500 moles/mole.

20. The method of claim 1 wherein the peptide load is increased to more than 1000 moles/mole.

21. The method of claim 1 wherein the favorable range is from 3.5 to 5.

22. The method of claim 1 further comprising, conjugating a plurality of each of various peptides with a wide range of pI to a carrier, measuring the peptide load for each of the peptides, and determining the favorable pI range for conjugation.

23. A peptide-carrier conjugate for eliciting immune response, which is made by the method of claim 1.

24. A peptide-carrier conjugate for eliciting immune response, the conjugate comprising,
an OMPC, and
a plurality of a modified peptide, each of the plurality of the modified peptide comprising a peptide and at least one anionic moiety or at least one neutral acyl group or both, wherein the at least one anionic moiety is covalently linked to the peptide; wherein the at least one neutral acyl group is covalently linked to an amino group of the peptide;
wherein the plurality of modified peptides are covalently linked to the OMPC through a plurality of a linker; wherein the modified peptide has a non-naturally occurring sequence.

25. The conjugate of claim 24 wherein the conjugate has a peptide load of more than 1000 moles/mole.

26. The conjugate of claim 24 wherein the modified peptides have a sequence selected from the group consisting of SEQ ID NO: 2, 4, 6, 8, 9, 10 and 11.

27. The conjugate of claim 24 wherein the amino group is the N-terminal amino group or the γ -amino group of lysine residue.

28. A vaccine for the prevention or amelioration of infection of a subject by a pathogen comprising a conjugate of Claim 23, an adjuvant, and a physiologically acceptable carrier.

29. A method of inducing an immune response in a mammal comprising the step of inoculating the mammal with an effective amount of a conjugate of Claim 28.

5 30. The method of Claim 29 wherein the mammal is a human.

31. A method for making a peptide-carrier conjugate, comprising:
modifying a first peptide to produce a second peptide so that the pI of the second peptide is in a favorable range or closer to the range than the pI of the first peptide; and
10 conjugating a plurality of the second peptide to a carrier to obtain a peptide-carrier conjugate;
wherein the peptide load, or the solubility of the conjugate, or both of them are increased by the modification; wherein when the modification is acetylation of N-terminal amino group, the N-terminal amino acid residue is not in a naturally occurring sequence.

15 32. The method of 31 wherein when the modification is acylation of N-terminal amino group, the N-terminal amino acid residue is not in a naturally occurring sequence.

33. The method of claim 31 wherein the carrier is selected from the group consisting
20 of OMPC (outer membrane protein complex of *Neisseria meningitidis*), BSA (bovine serum albumin), OVA (ovalbumin), THY (bovine thyroglobulin), KLH (keyhole limpet hemocyanin), and tetanus toxoid protein, HBs (Hepatitis B virus surface antigen protein), HBc (Hepatitis B virus core antigen protein), rotavirus capsid proteins, the L1 protein of the Human Papilloma Virus, VLP type 6, 11 or 16, and Dextran.

25 34. The method of claim 33 wherein the carrier is OMPC.

35. A peptide-carrier conjugate for eliciting immune response, which is made by the method of claim 31.

30 36. A method for determining the favorable pI range for conjugation comprising, conjugating a plurality of each of various peptides with a wide range of pI to a carrier, measuring the peptide load for each of the peptides, and determining the favorable pI range for conjugation.